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Acute mental stress elicits delayed increases in circulating inflammatory cytokine levels

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ABSTRACT

The influence of acute mental stress on cardiovascular responses and concentrations of inflammatory cytokines up to 2 h later was assessed in 12 subjects exposed to stress and in eight control subjects. Beat-by-beat recordings of finger blood pressure and heart rate were made at rest and during two behavioural tasks (colour–word interference and mirror tracing). Blood was drawn after adaptation and at 45 min and 2 h after the tasks, and assayed for interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α), interleukin-1 receptor antagonist (IL-1Ra), C-reactive protein (CRP) and haematocrit. Saliva was sampled periodically and assayed for free cortisol. The tasks were rated as stressful by the participants. The stress group showed significant increases in systolic and diastolic blood pressure (mean rises of 16.4 ± 12.3 and 12.6 ± 6.9 mmHg respectively) and heart rate (5.39 ± 5.3 beats/min); these values returned to baseline during the recovery period. The IL-6 concentration was increased by 56% at 2 h after the tasks ($P < 0.05$), while IL-1Ra was increased by 12.3% ($P < 0.01$). No changes in cardiovascular variables or cytokine concentrations were observed in the control subjects, and haematocrit did not change. The magnitude of blood pressure responses during tasks was correlated positively with the IL-6 concentration after 45 min ($r = 0.70$, $P < 0.05$), and with the IL-1Ra concentration after 2 h ($r = 0.63$, $P < 0.05$). Increases in TNF-α after 2 h were correlated with heart rate responses to tasks ($r = 0.66$, $P < 0.05$). Associations between IL-6 and IL-1Ra concentrations were also recorded. This study indicates that inflammatory cytokines respond to acute mental stress in humans with delayed increases, and suggest that individual differences in cytokine responses are associated with sympathetic reactivity.

INTRODUCTION

Animal experiments, clinical studies and epidemiological surveys implicate mental stress and other psychosocial factors in the aetiology of cardiovascular disease [1,2]. Cytokines, along with catecholamines and glucocorticoids, are among the principal messengers responsible for bi-directional communication between the central nervous system and the immune system [3]. Pro-inflammatory cytokines are thought to be involved in vascular endothelial dysfunction and the early stages of coronary atherosclerosis, as well as in the regulation of lipid and glucose metabolism [4,5]. It has been suggested that inflammatory cytokines such as interleukin-6 (IL-6), tumour necrosis factor α (TNF-α) and interleukin-1 (IL-1), along with the acute-phase reactant C-reactive protein

Key words: blood pressure, cardiovascular disease, cytokines, psychological stress.

Abbreviations: CRP, C-reactive protein; CV, coefficient of variation; IL-6, interleukin-6; IL-1Ra, interleukin-1 receptor antagonist; TNF-α, tumour necrosis factor-α.

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(CRP), mediate some of the effects of stress on human disease risk [6,7].

Studies in rodents indicate that a variety of severe stressors, such as foot shock, physical restraint and open-field exposure, stimulate increases in plasma IL-6 concentrations [8,9]. Immobilization stress also stimulates the hypothalamic expression of IL-1 mRNA [10], although less consistent effects have been observed with predator stress [11]. In humans, chronic stressors, such as anticipation of academic examinations, low control at work and caring for demented relatives, have been reported to result in increases in circulating levels of IL-1β, IL-6, TNF-α and IL-1 receptor antagonist (IL-1Ra) [12–15]. Raised serum concentrations of IL-6, IL-1 and IL-1Ra have also been recorded in some studies of clinical depression [16,17]. However, data concerning the impact of acute mental stress on inflammatory cytokines are inconclusive. In one study, no changes in the levels of IL-6, IL-1β, TNF-α or CRP were recorded in blood samples drawn immediately before and after performance of a stressful colour–word conflict task [18]. The explanation may be that cytokine responses are not immediate, since increases in concentration follow production from activated macrophages, endothelial cells, lymphocytes and smooth muscle cells. The impact of mental stress may therefore be delayed for minutes or even hours after stress. Consistent with this possibility, brief mental stress has been shown to stimulate vascular endothelial dysfunction up to 90 min after termination of the stressor [19]. Accordingly, we have assessed the influence of mental stress on cytokine levels in blood drawn 45 min and 2 h after the stress. Since mental stress may lead to transient decreases in blood volume, leading to increased haemoconcentration [20], the impact of changes in haematocrit was also measured. Subjective and behavioural data were collected, to confirm that the tasks did induce emotional stress. Along with IL-6 and TNF-α, we monitored IL-1Ra, since there is evidence that IL-1Ra is itself an acute-phase protein [21], and is increased under conditions of chronic emotional stress [22].

Another relevant factor is individual variation in the stress response. Increased cytokine production may not be a uniform response, but be related to the magnitude of acute cardiovascular reactions during stress exposure. Thus, in primate studies of social stress and atherogenesis, the development of coronary stenosis was greater in animals who showed high heart rate responses to a standardized acute challenge [23]. Maes et al. [13] recorded increased levels of IL-6, IL-1Ra and TNF-α in response to examination stress only in students who experienced a high level of subjective distress. In addition to measuring average cytokine levels before and after acute stress, we therefore computed correlations between the magnitude of acute cardiovascular reactions and subsequent cytokine levels. A separate comparison series of volunteers underwent the same measurement protocol in the absence of stress, to determine whether any non-specific changes in cardiovascular activity or cytokine levels could be observed.

METHODS

Subjects and procedures

The stress protocol was administered to 13 healthy volunteers (six men; seven women) aged 25–51 years (mean 40.9 years). They were of normal weight (body mass index 23.95 ± 3.7 kg/m^2), and three were cigarette smokers. The comparison series consisted of three men and five women aged 25–51 years (mean 34.6 years), with a body mass index of 22.2 ± 2.6 kg/m^2. The experiment was carried out in the afternoon, and the participants were instructed not to drink tea, coffee or caffeinated beverages, or to smoke, for at least 2 h before the study, and not to consume alcohol or exercise on the evening before or on the day of testing. The use of anti-inflammatory or anti-histamine medications for 48 h before testing was prohibited, and participants who had colds or other infections on the day of testing were rescheduled. The study was approved by the UCL/UCLH Committee on the Ethics of Human Research.

Mental stress was induced by two behavioural tasks commonly used in cardiovascular stress research. The first was a computerized colour–word interference task [24,25]. This involved the successive presentation of target colour words (e.g. green, yellow) printed in another colour. At the bottom of the computer screen were four names of colours, again printed in incongruous colours. The task was to press a computer key that corresponded to the position at the bottom of the screen of the name of the colour in which the target word was printed. The rate of presentation of stimuli was adjusted to the performance of the participant, to ensure sustained demands.

The second task was mirror tracing, involving the tracing of a star with a metal stylus that could only be seen in mirror image [26,27]. Each time the stylus came off the star, a mistake was registered and a loud beep was emitted by the apparatus (Lafayette Instruments Corp., Lafayette, IN, U.S.A.). Participants were told that the average person completed five circuits of the star in the time available, and were asked to give accuracy priority over speed for both tasks.

At the beginning of the test session, a venous cannula was inserted in the back of the non-dominant hand, and the blood pressure cuff was attached. The participant then rested for 20 min. Blood pressure and heart rate were recorded for the last 5 min of this period, after which a baseline blood sample was drawn, and a saliva sample was obtained for the measurement of free cortisol. The two behavioural tasks were then administered to experimental subjects in random order; five carried out

the colour word task followed by the mirror tracing task, while eight began with the mirror tracings. Each task lasted 5 min, during which blood pressure and heart rate were recorded continuously. The comparison subjects watched nature videos or read during this period. At the end of the tasks, a second blood sample (not for the assessment of cytokines) and a saliva sample were obtained. During the post-task recovery period, the participants sat quietly; 5-min recordings of blood pressure and heart rate were made at 15–20 min and 40–45 min during the post-task period, and saliva samples were obtained at 20 and 45 min. Further blood samples were drawn after 45 min and 2 h for assessment of cytokines and haematocrit.

Measurements

Cardiovascular measures

Systolic and diastolic blood pressure and heart rate were monitored continuously from the finger using the volume-clamp method with a Finapres model V instrument (TNO Biomedical Instrumentation, Amsterdam, The Netherlands). The Finapres finger cuff was positioned around the middle phalanx of the middle finger of the non-dominant hand, and an armrest was adjusted in height so that the cuff rested 10 cm below the base of the sternum.

Cytokine assays

TNF-α and IL-6 levels were measured using the high-sensitivity two-site ELISA from R&D Systems (Oxford, U.K.). The limit of detection of the human TNF-α assay was 0.10 pg/ml, with intra- and inter-assay coefficients of variation (CVs) of 6.9% and 8.4% respectively. For human IL-6, the limit of detection was 0.09 pg/ml, and intra- and inter-assay CVs were 5.3% and 9.2% respectively. IL-1Ra concentrations were determined using a commercial ELISA (R & D Systems). The assay has a limit of detection of 15 pg/ml, and inter- and intra-assay CVs of $< 10\%$. A sensitive, two-site ELISA for the determination of CRP was set up using antibodies from Dako Diagnostics (Ely, Cambs., U.K.). The assay range was 1.5–48 ng/ml, with inter- and intra-assay CVs of $< 10\%$.

Cortisol assays

Saliva samples were collected in Salivettes (Sarstedt, Leicester, U.K.), which were stored at $-30\,^{\circ}C$ until analysis. After defrosting, samples were centrifuged at 3000 rev./min for 5 min, and 100 μl of the supernatant was used for duplicate analysis involving a time-resolved immunoassay with fluorescence detection [28]. Haematocrit was assessed using a micro-haematocrit centrifuge (Hawksley Gelman, Lancing, W. Sussex, U.K.).

Subjective and behavioural measures

Behavioural performance of the tasks was measured in terms of the number of correct and incorrect responses to the colour–word task, and the number of circuits and errors on the mirror tracing task. At the end of each task, participants rated the difficulty of the task, task involvement, perceived performance, stress and feelings of control over the task, using a series of 7-point scales, ranging from 1 (low) to 7 (high).

Statistical analysis

Data were unsatisfactory from one participant in the stress protocol due to problems in blood collection, so the effects of stress were analysed in 12 subjects. Data were analysed using repeated-measures analyses of variance and co-variance, product–moment correlations and partial correlations. The analyses of systolic pressure, diastolic pressure and heart rate involved five 5-min trial periods (baseline, task 1, task 2, 20 min post-task and 45 min post-task). There were four trials in the cortisol analysis (baseline, immediate post-task, 20 min post-task and 45 min post-task). Haematocrit, IL-6, IL-1Ra, TNF-α and CRP were analysed over three trials (baseline, 45 min post-task and 2 h post-task). In the analyses of IL-6, IL-1Ra, TNF-α and CRP, the haematocrits recorded simultaneously were included as co-variates to guard against changes in concentration being secondary to alterations in haemoconcentration. Analyses were performed using SPSS. Data are represented as mean (S.D.).

RESULTS

Task performance and subjective responses

Participants attempted an average of 100.8 (S.D. 16.3) colour–word problems during the 5-min trial, and gave correct responses to 59.9 (14.3). An average of 5.67 (3.9) circuits were traced during the 5-min mirror tracing task, with 78.4 (60.4) errors. The subjective ratings indicated that the colour–word and mirror tracing tasks were both perceived as difficult (means 5.33 and 5.0 respectively), that participants felt moderately stressed (means 4.42 and 4.08), not in control (means 3.42 and 3.42), and that their performance was relatively poor (means 2.83 and 3.42). There were no significant differences between subjective ratings for the two tasks.

Cardiovascular responses

The stress group showed highly significant effects of trial (i.e. when measurements were taken) in the analyses of systolic pressure ($F = 5.67$, $P < 0.001$), diastolic pressure ($F = 6.35$, $P < 0.001$) and heart rate ($F = 13.4$, $P < 0.001$).

Table 1 Cardiovascular, neuroendocrine and cytokine responses to mental stress

Values are means (S.D.). Repeated-measures multivariate ANOVA (MANOVA) was used to assess the effect of trial (*F* and *P* values); ns, not significant.

Parameter	Baseline	Task 1	Task 2	Immediate post-task	20 min post-task	45 min post-task	2 h post-task	MANOVA trial effect	
								F	*P*
Systolic pressure (mmHg)	124.7 (19.6)	140.6 (27.9)	139.1 (28.4)		132.6 (19.6)	128.6 (17.4)		5.67	< 0.001
Diastolic pressure (mmHg)	74.5 (14.2)	85.7 (19.7)	85.6 (19.7)		82.0 (11.3)	78.1 (15.3)		6.35	< 0.001
Heart rate (beats/min)	71.0 (9.4)	77.0 (8.1)	75.9 (8.8)		64.0 (8.1)	67.4 (8.3)		13.4	< 0.001
Cortisol (nmol/l)	7.54 (3.3)			6.55 (2.2)	5.05 (1.7)	4.12 (1.3)		12.2	< 0.001
Haematocrit (%)	39.1 (4.6)			38.9 (4.5)		38.4 (4.4)	38.4 (5.9)	0.36	ns
IL-6 (pg/ml)	1.67 (0.65)					1.73 (0.92)	2.61 (2.1)	4.73	0.023
IL-1Ra (pg/ml)	216.4 (78)					225.6 (77)	243.1 (92)	7.11	0.006
TNF-α (pg/ml)	1.12 (0.54)					1.12 (0.39)	1.16 (0.48)	0.03	ns
CRP (ng/ml)	2.29 (0.91)					2.35 (0.94)	2.26 (1.0)	0.72	ns

Table 2 Cardiovascular, neuroendocrine and cytokine responses – control series

Values are means (S.D.). Repeated-measures multivariate ANOVA (MANOVA) was used to assess the effect of trial (*F* and *P* values); ns, not significant.

Parameter	Baseline	Task 1	Task 2	Immediate post-task	20 min post-task	45 min post-task	2 h post-task	MANOVA trial effect	
								F	*P*
Systolic pressure (mmHg)	128.6 (12.1)	128.0 (13.1)	130.0 (11.4)		129.6 (11.3)	131.3 (10.7)		0.41	ns
Diastolic pressure (mmHg)	71.0 (6.0)	71.7 (7.3)	73.0 (5.9)		72.8 (8.1)	73.0 (6.6)		1.13	ns
Heart rate (beats/min)	65.8 (9.7)	64.1 (9.6)	64.8 (9.0)		65.2 (8.1)	62.6 (7.5)		3.48	0.022
Cortisol (nmol/l)	7.02 (4.2)			5.59 (2.9)	3.89 (1.6)	3.88 (2.3)		8.32	0.003
Haematocrit (%)	36.5 (3.1)			35.3 (2.0)		36.7 (1.9)	36.8 (1.7)	1.43	ns
IL-6 (pg/ml)	1.07 (0.56)					1.14 (0.74)	1.62 (0.72)	3.60	ns
IL-1Ra (pg/ml)	176.7 (49.9)					217.3 (115.9)	235.6 (925)	1.04	ns
TNF-α (pg/ml)	1.56 (0.59)					1.13 (0.47)	1.27 (0.64)	3.67	ns
CRP (ng/ml)	2.29 (0.73)					2.48 (0.81)	2.56 (0.85)	0.37	ns

As can be seen in Table 1, blood pressure and heart rate increased during the behavioural tasks, returning to baseline levels during the post-task recovery period. The average increases were > 15 mmHg for systolic pressure and > 10 mmHg for diastolic pressure.

No significant changes in systolic or diastolic pressure were recorded over time in the comparison group (Table 2). There was an effect of trial in the analysis of heart rate ($F = 3.48$, $P = 0.022$), due to a decrease between the 20 and 45 min post-task trials.

Cortisol and haematocrit

Saliva free cortisol levels at baseline averaged 7.54 (3.3) nmol/l in the stress group and 7.02 (4.2) nmol/l in the comparison group. There were significant effects of trial in both groups ($F = 12.2$ and $F = 8.32$ respectively; $P < 0.005$), due to progressive reductions in cortisol over time. There was no increase in cortisol following behavioural tasks in the stress group (Table 1). Thus the mental stressors used in this study were not effective in stimulating increases in salivary cortisol. Haematocrit did not change significantly over time in either group.

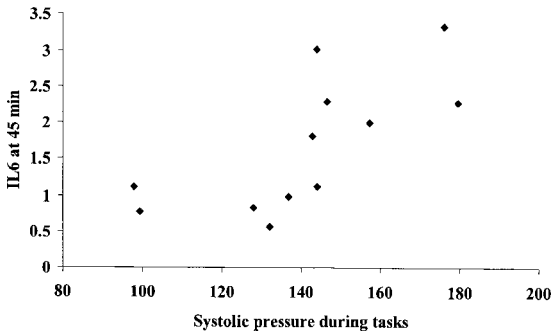

Figure 1 Scatter plot showing the association between systolic pressure (mmHg) during tasks and IL-6 (pg/ml) measured 45 min after tasks for individuals in the stress group ($r = 0.70$)

Cytokine responses

There were significant main effects of trial in the stress group in the analyses of plasma concentrations of IL-6 ($F = 4.73$, $P = 0.023$) and IL-1Ra ($F = 7.11$, $P = 0.006$). As shown in Table 1, IL-6 increased between baseline and

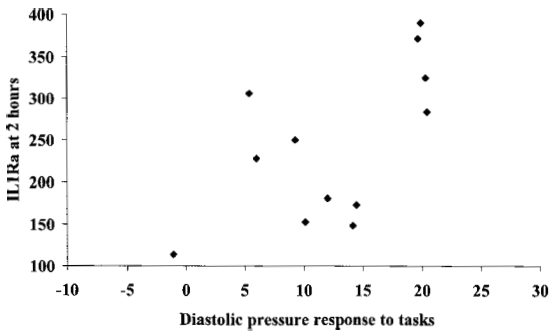

Figure 2 Scatter plot showing the association between diastolic pressure response to tasks (mmHg) and IL-1Ra (pg/ml) measured 2 h after tasks for individuals in the stress group ($r = 0.63$)

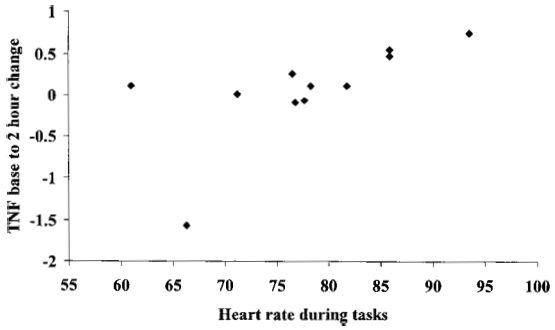

Figure 3 Scatter plot showing the association between heart rate (beats/min) during tasks and changes in TNF-α (pg/ml) between baseline and 2 h after tasks for individuals in the stress group ($r = 0.66$)

2 h post-task by an average of 56%, while IL-1Ra increased by 12.3%. There were no significant changes in TNF-α or CRP in the stress group. In the comparison group, the effects of trial did not approach significance for any variable.

Associations between cardiovascular stress reactivity and cytokine responses

The systolic pressure attained during behavioural tasks was positively associated with IL-6 concentration measured at 45 min post-task ($r = 0.70$, $P = 0.011$). This effect remained significant after partialling out systolic pressure measured at the time of the 45 min blood sample (partial $r = 0.71$, $P = 0.032$), and so reflects a relationship between IL-6 responses and the magnitude of stress-induced increases in blood pressure. The association is plotted in Figure 1. IL-6 levels in the comparison group were not correlated with cardiovascular measures.

The levels of IL-1Ra at 45 min post-task, and more markedly at 2 h post-task, were positively correlated with the increase in diastolic pressure between baseline and task periods ($r = 0.55$ and $r = 0.63$ respectively; $P = 0.062$ and $P = 0.034$ respectively). The relationship is plotted in Figure 2, and shows that IL-1Ra levels were greater 2 h after tasks in those individuals who reacted to mental stress with larger increases in diastolic pressure. Associations were not significant in the comparison group.

There were also significant relationships between cardiovascular stress reactions and TNF-α levels. In particular, the change in TNF-α between baseline and 2 h post-task was positively correlated with heart rate during the tasks ($r = 0.66$, $P = 0.028$). This effect is plotted in Figure 3. Subjects whose heart rate was higher during the tasks tended to show increases in TNF-α between baseline and 2 h. It can be seen from Figure 2 that one subject produced a substantial decrease in TNF-α in comparison with all others in the stress group. When this individual was omitted from the analysis, the correlation between the change in TNF-α levels and heart rate during tasks increased ($r = 0.84$, $P = 0.002$). Again, there were no effects in the comparison group. CRP was not correlated with cardiovascular stress reactivity at any point in the study.

Associations between the cytokine responses

Statistical correlations were sought between levels of TNFα, IL-6, CRP and IL-1Ra, at baseline and after the stress test, as evidence suggests that the production of these cytokines is inter-regulated. There were no significant associations between levels of TNF-α and IL-6

Table 3 Effects of mental stress on the correlation between IL-6 and IL-1Ra concentrations

Values shown are Pearson correlation coefficients (r) and two-tailed significance (P).

	IL-1Ra					
	Baseline		45 min post-task		2 h post-task	
IL-6	*r*	*P*	*r*	*P*	*r*	*P*
Baseline	0.42	0.18	0.46	0.13	0.47	0.13
45 min post-task	0.62	0.03	0.63	0.03	0.58	0.05
2 h post-task	0.65	0.02	0.63	0.03	0.55	0.06

at baseline, or at any of the other time points studied. However, while the association between IL-6 and IL-1Ra was not significant at baseline, these correlations reached significance 45 min after the stress test (Table 3).

None of the correlations between levels of TNFα, or IL-6, and CRP were significant during the course of the study.

DISCUSSION

The behavioural tasks in this study elicited moderate acute stress responses, as indicated by subjective reports and cardiovascular measures. The increases in blood pressure and heart rate were comparable with those recorded previously using these stimuli [25,27]. However, saliva free cortisol did not increase following the tasks, contrary to expectation. Increases in cortisol levels generally take place some 10–30 min following stress, returning to baseline after about 1 h [29]. It is possible that the stressors were not sufficiently intense to stimulate detectable cortisol responses. Another factor is that cortisol may have already risen in response to cannulation, since blood sampling is a potent stressor for many individuals. Both stress and comparison subjects showed a similar pattern, suggesting a common response to the measurement procedure.

We observed significant increases in IL-6 and IL-1Ra concentrations in the stress group, and these were higher at 2 h than at 45 min post-stress. With only two sampling points, we do not know whether the peak cytokine response occurred between 45 min and 2 h, or whether concentrations would have continued to rise after the end of the experiment. No such elevations in the concentrations of either IL-6 or IL-1Ra were apparent in the control group, suggesting that the cannulation did not cause local increases in cytokine concentrations during the course of the study. The delayed response in the stress group may explain why increases in levels of inflammatory cytokines have not been recorded consistently following acute mental stress. Dugué et al. [18] administered a 25 min colour–word conflict task, but measured cytokine levels immediately after the task. In contrast, Ackerman et al. [30] observed that mitogen-stimulated production of TNF-α was increased 5 min after a speech task, but that further increases emerged after 60 min. Similarly, lipopolysaccharide-induced production of IL-6, but not TNF-α, was raised in blood drawn immediately after a stressful speech task [31]. Speech tasks typically generate greater stress reactions than the types of behavioural tasks used in the present study, since they are socially evaluative and more immediately challenging to the participant's self-presentation. However, the mechanisms underlying increases in circulating cytokines and production by exogenously stimulated lymphocytes may differ.

Haematocrit was measured at the same time points as cytokines, in order to control for the effects of changes in haemoconcentration. Reductions in blood volume due to haemodynamically mediated compartmental transvascular shifts of plasma into the interstitial space or tissues have been observed in a number of laboratory mental stress studies, and may account in part for acute increases in the concentration of lipids [20,24] and in lymphocyte subset redistribution [32]. However, no significant change in haematocrit was recorded in either experimental group. Other studies have recorded increases in haematocrit with severe, but not moderate, mental stress [33], and the stimuli utilized in the present study may not have been sufficiently intense to induce changes.

The mechanisms governing the immune system and cytokine release are complex, but one factor that may have been particularly relevant in the present study is activation of the sympathetic nervous system. In rodents, sympathetic nervous system activation during stress was associated with elevated plasma IL-6 levels [34]. Furthermore, adrenaline has been shown to induce an acute rise in plasma IL-6 levels [35]. These observations are also consistent with a possible role for catecholamines as mediators of the exercise-induced elevation of plasma IL-6 levels [36]. It has been demonstrated that isoprenaline infusion elicits increases in systemic levels of IL-6 [37].

Cytokines regulate the expression and release of other cytokines, and therefore their activities should be considered as a network. TNF-α induces the release of IL-6, and this cytokine has then been shown to induce the release of IL-1Ra and CRP [38–40]. IL-1Ra in turn inhibits IL-6 release, probably facilitating resolution of the inflammatory response locally and at remote sites [41]. The lack of any significant changes in TNF-α levels in the present study may be due to the fact that the effects of TNF-α are mainly autocrine or paracrine, unlike those of IL-6, which is an endocrine cytokine. We observed significant associations between levels of IL-6 and IL-1Ra after stimulation of their release by mental stress. At 45 min post-stress, both cytokines were elevated to a similar magnitude. Further rises in their levels, especially for IL-1Ra, were observed after 2 h, compared with baseline. While previous studies have demonstrated an association between CRP and IL-6 in response to acute stress, we were unable to detect an increase in CRP levels. IL-6 and IL-1Ra are released by circulating mononuclear cells, and the magnitude and duration of the stimulus provided by acute stress may be adequate to elicit this response. Conversely, this stimulus may be inadequate to cause IL-6 induction of the hepatic acute-phase response and therefore the release of CRP.

Another important finding in the present study was that the responses of inflammatory cytokines measured 45 min and 2 h post-stress were correlated with cardiovascular responses during the tasks themselves. All

three cytokines (IL-6, IL-1Ra and TNF-α) showed significant associations with cardiovascular reactivity, although with varying time courses. These findings suggest that individual differences in the magnitude of sympathetic nervous system activation may be important. There is accumulating evidence that mental stress does not exert uniform effects, and that variations between people in the magnitude of sympathetic responses are significant. Thus the degree of increase in blood pressure or heart rate in response to standardized tasks has been associated with the extent of left ventricular mass in young people and adults [42,43], and with carotid atherosclerosis in middle-aged men [44]. In patients with coronary heart disease, mental-stress-induced myocardial ischaemia is more frequent in people who show exaggerated blood pressure and heart rate responses to mental stress tests [45]. Individual differences in cardiovascular stress responsivity are also correlated with the magnitude of acute increases in the number of circulating natural killer cells [46].

Evidence for the involvement of inflammatory cytokines in atherogenesis and cardiovascular health is growing. Elevated levels of circulating IL-6 have been shown to predict future myocardial infarction in healthy men and women [47,48], and mortality in an older cohort [49]. The plasma concentration of TNF-α is raised in cardiac patients who experience recurrent coronary events [50], while outcome in patients with unstable angina is poor among individuals with elevated IL-1Ra and IL-6 levels [51]. The observation that the cytokines are responsive to mental stress suggests that they may be involved in the pathways through which psychosocial factors influence cardiovascular disease risk.

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